Cancer Research Takes Flight:

Wnt Signaling in Development and Disease

What do cancers have in common with fruit fly wings? Wnts. The very name of the Wnt (pronounced /wint/) family of secreted signaling molecules proclaims its dual history in developmental biology and cancer research. The "w" comes from wingless, a gene necessary for the proper development of the fruit fly body plan. The "nt" comes from Int oncogenes, first identified near sites of integration of the mouse mammary tumor virus. There are 19 Wnt genes in the human genome. Their tight regulation orchestrates development both embryonically and into adulthood; their misregulation contributes to multiple cancers. The merging of these two lines of research, which is now more than 25 years in the making, has been a boon for both fields.

Terry Yamaguchi, Ph.D., Head of the Cell Signaling in Vertebrate Development Section in CCR's Cancer and Developmental Biology Laboratory, came to Wnt signaling from developmental biology. His research has taken him from an interest in the late stages of muscle differentiation steadily backwards to the role of Wnts in the earliest steps of cell specification from embryonic stem cells. Now he hopes to define the key molecular events that govern the fate of stem cells in embryonic development and to apply that knowledge to understanding how stem cells contribute to adult tissues normally as well as how abnormal signaling gives rise to cancers.

Location, Location, Location

The mantra of "location, location, location" is as critical for determining cell fate during development as it is for setting the value of real estate, but location works its magic in development through the much more complicated process of gene regulation. Embryonic stem cells are originally pluripotent—capable of developing into almost any cell type, but their fates are gradually refined as they interact with their local environment. The pluripotent embryonic stem cells soon give rise to three germ layers—ectoderm, mesoderm, and endoderm—which give rise to specific tissues. Gradients of secreted signaling molecules activate distinct gene expression programs in the cells of each germ layer, which in turn regulate the cells' interaction with the gradients of signaling molecules they encounter.

(*Photo:* R. Baer)

Terry Yamaguchi, Ph.D. *(right)*, with Postdoctoral Fellow Bill Dunty, Ph.D. *(left)*, and technician Kirstin Biris *(center)*.

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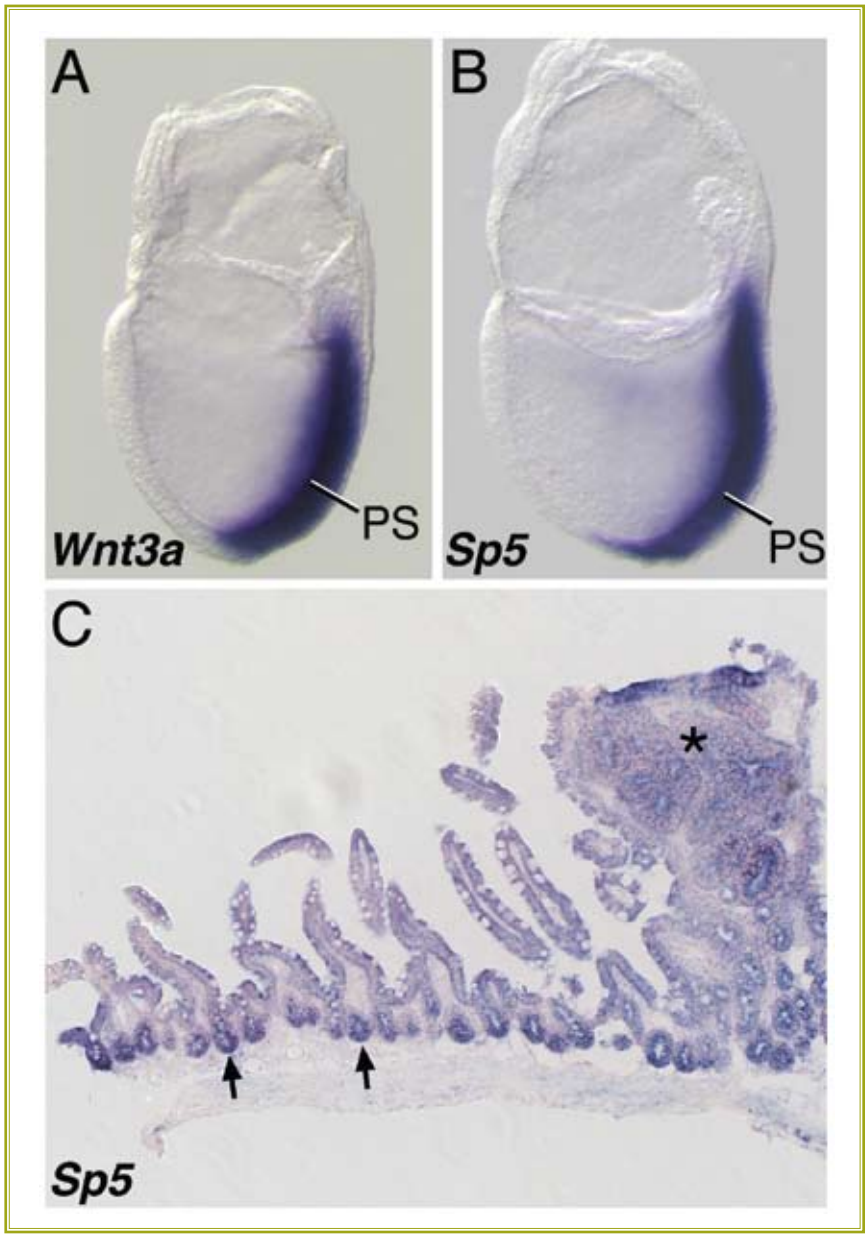
The trick throughout development is to create signals that are sufficiently restricted in time and space that they balance the production of more stem cells (proliferation) with the production of specific cell types (differentiation) to produce exactly and only as many cells as necessary for a specific tissue, a concept known as stem cell homeostasis.

The 19 Wnt ligands are generally expressed in patterns that are tightly regulated in time and space throughout development. Mutation of these genes usually results in dramatic developmental defects, although there appears to be some redundancy in the system so that a single *Wnt* mutation may leave an embryo seemingly unimpaired. Without *Wnt3a*, for example, the entire trunk and tail mesoderm fails to form. To similarly "disappear" the lungs, however, requires the double mutation of *Wnt2* and *Wnt2b*.

"The primitive streak," described Yamaguchi, pointing to a dark purple line in a micrograph of an eight-day old embryo, "is a source of many secreted signaling molecules, including Wnt3a, which can pattern the entire anteroposterior axis." Whereas it was once believed that the primitive streak was simply a point through which cells transit as they become the mesoderm of the trunk and tail, it now seems that the primitive streak is also a source of stem cells that give rise to the germ layers. "One of the main hypotheses that we are pursuing is that Wnt3a in the primitive streak is required for the maintenance of mesodermal stem cells."

Through a series of genetic experiments, published in the January 2008 issue of *Development*, Yamaguchi, Postdoctoral Fellow Bill Dunty, Ph.D., and their colleagues have formally demonstrated that Wnt3a works through the well-studied canonical β-catenin pathway to support mesodermal stem cells. β-catenin is normally maintained at low levels in the cellular milieu by the APC/axin complex, which steadily consigns β-catenin to degradation. Wnt signaling sequesters some of the components of the degradation complex, resulting in increased levels of β-catenin, which can then make its way to the nucleus to activate the transcription of a number of target genes.

"The main point of this pathway from our perspective is that its stimulation activates a transcriptional program of gene expression. One of the big goals in the lab is to identify what this pathway is doing in the early embryo and identify the target genes through a transcriptional profiling approach." By looking at the gene expression patterns in different Wnt3a and β-catenin mutant mice, Yamaguchi's team has identified 62 genes that may be regulated by this pathway. Some, like *Sp5* and *Axin2*, are known targets of the Wnt/β-catenin system in other contexts, some are known oncogenes like *Myc*, and others



(*Image: T. Yamaguchi, CCR*)

Sp5 is an example of a Wnt3a/β-catenin target gene that is expressed in primitive streak (PS) stem cells of the mouse embryo (*panels A and B*) and in adult intestinal crypt stem cells (*panel C, arrows*) and adenomas (*asterisk*).

appear to be completely novel. Kristin Biris, a technician in Yamaguchi's group, is using *in situ* hybridization to determine where these Wnt3a target genes are expressed in early embryos.

One of the most interesting target genes they have studied so far is *Mesogenin 1*, which is itself not only directly activated by Wnt3a signaling but also appears to operate in a feedback loop to inhibit Wnt3a signaling. Such an inhibitory feedback mechanism could allow high concentrations of Wnt3a, as found in the primitive streak, to support mesodermal stem cell renewal, whereas the effects of lower concentrations of Wnt3a would be inhibited by *Mesogenin 1* feedback, turning a gradient of Wnt3a into a threshold that supports either proliferation or the differentiation of mesodermal stem cells.

Into the Crypt

High concentrations of Wnts are not confined to embryonic development. They reappear, among other places, in the adult intestine, where they regulate the intestinal stem cell niche. "Wnts are so conserved, and their expression is so closely associated with stem cell populations, we believe that what we learn from the early embryo may be generally applicable to other stem cells in the adult," said Yamaguchi. He and his colleagues are now beginning to put that belief to the test.

The adult intestine is coated with a single layer of epithelial cells that are responsible for digestion and absorption, as well as for providing a barrier against pathogens. These epithelial cells, of which there are four major types, are replaced every 4–5 days by a process of cellular renewal. The stem cells that give rise to these new cells are found in pockets of cells called the intestinal crypt. Deep in the crypt, new cells are born and mostly migrate upwards and away from the source of their renewal, up into finger-like protrusions of the intestine called villi. Three days after their cellular identity or fate is sealed, they reach the tip of the villus, self-destruct, and are shed away to be replaced by younger cells.

It turns out that Wnt signaling controls this process of self-renewal, operating as a kind of master switch between proliferation of stem cells and differentiation into epithelial cells. Several studies have shown that Wnt-target gene expression occurs in a gradient that is strongest at the base of the intestinal crypt and weakens further away. And loss of β-catenin, the key transducer of Wnt signaling, dramatically reduces intestinal cell proliferation.

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Yamaguchi and his colleagues have compared the gene expression patterns they observed in the embryonic mesoderm with that of the adult intestinal crypt and found a remarkable 60 percent of the Wnt-target genes that they identified in mesodermal stem cells are also found in the adult intestinal stem cells. Identifying these genes is the first step in establishing the critical molecular network that is responsible for stem cell maintenance, whether in the embryo or in the adult. Functional follow-up studies will be necessary to establish their roles in cellular renewal (see "The Power of Embryonic Stem Cells").

Development Gone Awry

The precise regulation of development, once tampered with, can quickly give rise to abnormal growth that is the hallmark of cancer. "From my developmental perspective, cancer is developmental signaling gone awry," explained

(*Photo: R. Baer*)

Terry Yamaguchi, Ph.D. *(left)*, with Postdoctoral Fellow Ravi Chalamalasetty, Ph.D. *(right)*.

Yamaguchi. Already in 1989, mutations of the gene *Adenomatous polyposis coli (APC)* were found in patients with familial adenomatous polyposis (FAP) and in sporadic colorectal cancers before it was understood that *APC* was a critical component of the Wnt signaling pathway. Since then, several other mutations in the canonical Wnt signaling cascade have been associated with cancers.

Hans Clevers, M.D., Ph.D., and colleagues at the Utrecht University Medical Centre, The Netherlands, showed in a paper published in *Nature* this year that deleting *APC* in long-lived intestinal crypt stem cells—but not in differentiated cells migrating away from the intestinal crypt—leads to intestinal adenomas. The transformed stem cells appear to remain in the crypts, steadily fueling growth of the adenomas, and they may represent one of the best examples of a true cancer stem cell.

Studying the same Wnt3a-target genes that he originally identified in embryonic mesoderm, Yamaguchi has shown that 40 percent of these genes are expressed in intestinal adenomas. "So, we're asking whether any of these genes are required downstream of Wnt signaling for tumor formation." Specifically, in a mouse model of intestinal adenomas in which the β-catenin pathways are constitutively active, Yamaguchi and his colleagues are asking whether they can reduce the tumor burden by knocking out some of the Wnt-target genes. Conversely, they are also trying to make transgenic mice that overexpress individual target genes specifically in the intestinal epithelium to ask whether they alone are sufficient to form tumors.

"The connection of the Wnt pathway to human cancer is very strong—mutations in this pathway are associated with 85–90 percent of human colorectal cancers. And this case is one where the animal model, although not perfect, is quite good for human cancer. The molecular mutations in both cases are essentially the same. Thus, we have a great opportunity to apply what we know from normal biology to an animal model of cancer."

For more information about Dr. Yamaguchi's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=yamaguchi.

The Power of Embryonic Stem Cells

Embryonic stem cells are not only part of the biology fueling Terry Yamaguchi, Ph.D.'s, intellectual curiosity, but they are also a means to satisfy that curiosity. As a graduate student, Yamaguchi first exploited the ability of embryonic stem cells to differentiate *in vitro* to identify the growth factors important for the specification of mesodermal fates. His work led to the discovery of a family of receptors that regulates the stem cells that give rise to blood and blood vessels. Now Yamaguchi is an enthusiastic adopter of a powerful embryonic stem cell system generated by Michael Kyba, Ph.D.'s, group at the University of Minnesota.

While *in vivo* studies are critical to developmental biology, testing biochemical mechanisms is difficult to do in an embryo of only a few thousand cells. "We wanted to move away from an embryo to a stem cell population we could manipulate *in vitro*," explained Yamaguchi. Dr. Kyba had recently developed an inducible system for expressing genes of interest in embryonic stem cells in a consistent and reliable way. The gene is always integrated at the same genetic locus, and it is induced by the application of doxycycline.

"These [embryonic stem] cells are a great compromise—we can do so much manipulation genetically and biochemically *in vitro*—and the sky is the limit because the efficiency of targeting is so good," enthused Yamaguchi. In their laboratory, and with these cells, the probability of a successfully engineered cell is almost 95 percent for any gene of interest. They are, therefore, able to screen all of the Wnt3a target genes they identify by transcriptional profiling for functional activity and biochemical interactions. Postdoctoral Fellow Ravi Chalamalasetty, Ph.D., is using this approach to identify the targets of *Mesogenin 1* (see main text).

Once stable embryonic stem cell lines are engineered with the inducible gene of interest, these cells can be taken back to the embryo to produce a transgenic mouse. Additionally, once the culture conditions are solved, these cell lines can be differentiated *in vitro* into any cell type (e.g., a heart cell or a neuron or, maybe one day, an intestinal stem cell). "For me," said Yamaguchi, "the possibilities are endless with these embryonic stem cells."